Oral Microbiol Immunol 1994: 9: 364–371
Printed in Denmark · All rights reserved

Copyright © Munksgaard 1994

Oral Microbiology and Immunology
ISSN 0902-0055

Kinetics of lactose-reversible coadhesion of *Actinomyces naeslundii* WVU 398A and *Streptococcus oralis* 34 on the surface of hexadecane droplets

R. P. Ellen[1], H. Veisman[1,2]
I. A. Buivids[1], M. Rosenberg[1,3]

[1]Faculty of Dentistry, University of Toronto, [2]Faculty of Dentistry, University of Western Ontario, London, Ontario, Canada, [3]Maurice and Gabriela Goldschleger School of Dental Medicine, Tel-Aviv University, Ramat-Aviv, Israel

Ellen RP, Veisman H, Buivids IA, Rosenberg M. Kinetics of lactose-reversible coadhesion of Actinomyces naeslundii *WVU 398A and* Streptococcus oralis *34 on the surface of hexadecane droplets.*
Oral Microbiol Immunol 1994: 9: 364–371. © Munksgaard, 1994

Most investigations of mechanisms accounting for intergeneric coaggregation have emphasized stereospecific rather than nonspecific interactions. The purpose of this investigation was to determine the relative importance of lectin-carbohydrate and nonspecific hydrophobic and ionic interactions, using a model based on strains with one of the most well understood specific coaggregation mechanisms, the lactose-reversible coaggregation of *Actinomyces naeslundii* and *Streptococcus oralis*. The kinetics of coadhesion and desorption of coadherent bacteria were studied using *S. oralis* 34 bound to hexadecane droplets as an affinity support for the adhesion of *A. naeslundii* WVU 398A. Light, confocal microscopy and transmission electron microscopy confirmed that *A. naeslundii* cells adhered only to the *S. oralis* cells, not to exposed hexadecane between the streptococci. Coadhesion was inhibited by lactose concentrations as low as 2.0 mM. The rate of coadhesion was halved at 60 mM lactose. The hydrophobicity inhibitors bovine serum albumin and defatted bovine serum albumin and the salts LiCl and KCl failed to inhibit coadhesion in the hexadecane assay, and bovine serum albumin also failed to inhibit coaggregation in a bacterial aggregation assay on glass slides. High concentrations of the salts achieved a 50% rate decrease in *A. naeslundii* adhesion to the *S. oralis*-coated droplets only when they were combined with >20 mM lactose. Sodium dodecyl sulfate (SDS) and Tween 20 inhibition was tested by the slide coaggregation assay because they tended to emulsify the droplets; SDS was inhibitory. Lactose selectively desorbed *A. naeslundii* from *S. oralis*-coated droplets at low concentrations equivalent to those that inhibited coadhesion. Neither LiCl nor KCl desorbed *A. naeslundii* from the droplets, even at 500 mM. At low concentrations, SDS but not Tween 20 eluted both *A. naeslundii* and *S. oralis* from the droplets. Although the SDS results might suggest a degree of cooperative charge interactions, the results support the hypothesis that stereospecific, β-galactoside-sensitive interactions have a much greater impact than nonspecific interactions on the coadhesion of *A. naeslundii* and *S. oralis*.

Key words: coadhesion; kinetics; specificity; hydrophobicity; *Actinomyces naeslundii*; *Streptococcus oralis*

Richard P. Ellen, Department of Periodontics, University of Toronto, Faculty of Dentistry, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6

Accepted for publication December 20, 1993

Coadhesion of dissimilar species influences the sequential establishment of bacteria in dental plaque and helps foster their retention as a biofilm on teeth (6). Experimentally, it is most often studied *in vitro* by mixing 2 bacterial suspensions in the absence of a supporting surface and observing the degree to which they coaggregate during a timed assay. Our laboratory and others have developed *in vitro* assays in which one species is added to cells of another which have first been adsorbed as a monolayer on a surface (1, 8, 11, 15, 16, 18). Such assays allow investigations of the rate of coadhesion and the effect of inhibitors on the kinetics of coadhesion. They are particularly useful for simulat-

ing the order in which the selected species usually re-establish on cleaned teeth and for conducting experiments when conventional coaggregation assays are difficult to assess because one or both of the paired species self-aggregates (9, 10, 16).

Studies of coaggregation mechanisms have most often emphasized the stereospecificity of interacting molecules on the surfaces of the pairs. Most pairs of oral bacteria that coaggregate have been reported to interact by lectin-carbohydrate recognition (6,7). With few exceptions (13, 15), neither the avidity of coaggregation nor the potential contribution of nonspecific interactions to the stability of developing coaggregates has been addressed quantitatively. We have recently introduced an assay by which the rate of coadhesion on the surfaces of hexadecane droplets and the influence of inhibitors of hydrophobicity can be measured. Its utility in studying the kinetics of *Porphyromonas gingivalis-Actinomyces viscosus* interactions and in documenting the effects of proteases and other proteins on coadhesion has been demonstrated (5, 15). However, using it to determine the relative importance of specific versus nonspecific interactions is currently premature with these 2 species, because specific mechanisms underlying their mutual recognition have not been well characterized at the molecular level.

The most well documented specific coaggregation interaction among oral bacteria is the β-galactoside-inhibitable reaction between *Actinomyces naeslundii* and oral streptococci such as *Streptococcus oralis* (6, 19). During plaque formation *in vivo*, the adsorption of *S. oralis* to salivary pellicle on teeth or simulations of tooth surfaces typically precedes that of *A. naeslundii* (3, 12). Therefore, we selected a pair of well characterized, coaggregating strains to study the relative significance of stereospecific and electrostatic or hydrophobic interactions in establishing and disrupting their coadhesion. The test hypothesis was that galactoside-inhibitable and -reversible interactions have a far greater impact than nonspecific forces on the kinetics of *A. naeslundii-S. oralis* coadhesion.

Material and methods

Bacterial strains and culture conditions

A. naeslundii WVU 398A was originally obtained from M. A. Gerencser, West Virginia University, and maintained in the lyophilized culture collection at the University of Toronto. Stocks were maintained at 4°C on brain heart infusion agar slants (Difco Laboratories, Detroit, MI). For experiments, 10-ml tryptic soy broth (Difco Laboratories) cultures were grown for 48 h from inocula off the slants, and these were used at a 1 : 100 dilution to inoculate broth culture tubes for a subsequent 48-h growth period in an atmosphere of 80% N_2, 10% CO_2 and 10% H_2. Cells were harvested by refrigerated centrifugation and washed twice in modified coaggregation buffer (7) containing 121.1 mg TRISMA-Base ((Sigma Chemical Co., St. Louis, MO) 14.7 mg $CaCl_2 \cdot H_2O$, 20.3 mg $MgCl \cdot 6H_2O$, 8.77 g NaCl, per liter distilled H_2O, pH 8.0). Coaggregation buffer suspensions were prepared in glass test tubes at various optical densities that were corrected for nonlinearity, ranging downward from 1.2 at 550 nm (OD_{550}) in a model 350 spectrophotometer (G. K. Turner Associates, Palo Alto, CA). The optimum OD_{550} for coadhesion assays was found to be 1.0 and for slide coaggregation assays 1.2.

S. oralis 34 was originally obtained from R. J. Gibbons, Forsyth Dental Center and maintained lyophilized. Stock cultures were maintained on tryptic soy agar plates containing 5% defibrinated sheep blood. For experiments, *S. oralis* was grown aerobically in tryptic soy broth for 24 h and then inoculated at 1 : 100 dilution for an additional 24 h growth. Cells were washed and suspended in coaggregation buffer as described above.

Adhesion to hexadecane

To confirm their innate hydrophobicity, the adhesion of *A. naeslundii* 398A and *S. oralis* 34 to hexadecane was measured prior to their use in coadhesion assays (15). Cell suspensions ($OD_{550} = 1.2$) were distributed in 2.0-ml aliquots into borosilicate glass tubes; 0.15 ml *n*-hexadecane was added to each tube. The suspensions were mixed at the highest setting on a Vortex-Genie (Scientific Industries, Bohemia, NY) for 4 consecutive 1-min periods. Following phase separation, the change in turbidity of the aqueous phase was measured. For *S. oralis* 34, the contents of the test tubes were pooled, and the aqueous phase removed, yielding hexadecane droplets coated with streptococci. The optimum OD_{550} to produce *S. oralis* 34-coated hexadecane droplets for coadhesion experiments was found to be 1.0. The *S. oralis*-coated droplets were washed by gently swirling them with fresh coaggregation buffer as the aqueous phase to ensure their uniform distribution prior to dispensing into test tubes.

Coadhesion and coaggregation assays

Coadhesion of *S. oralis*-coated hexadecane droplets and *A. naeslundii* 398A followed the methods for *P. gingivalis-A. viscosus* pairs that we had published previously (15). Briefly, to 1.0 ml of *A. naeslundii* suspension, OD_{550} 1.0, was added 0.3 ml of coaggregation buffer, 0.2 ml of inhibitor and 0.5 ml of *S. oralis*-coated droplets. When a second inhibitor was added, volumes of buffer and inhibitor were varied accordingly to maintain a final volume of 2.0 ml. Controls included: 1) tubes containing *n*-hexadecane free of *S. oralis*; 2) tubes containing only *S. oralis*-coated droplets in buffer, with or without inhibitors, and 3) tubes containing *S. oralis*-coated droplets and *A. naeslundii* as a positive control when the effect of inhibitors was determined. All tubes were incubated at ambient temperature with gentle rotation at a fixed 40° incline in a multipurpose rotator (Scientific Industries). At 2-min intervals, phases were allowed to separate, and the turbidity of the lower phase was measured directly.

Three microscopy methods were used to examine the *S. oralis*-coated droplets, with and without adherent *A. naeslundii* cells. 1) For conventional light microscopy, the droplets were exposed to 1.0 ml of aqueous solution of safranin diluted 1/10, allowed to rotate for 15 min and washed 5 times in coadhesion buffer. *A. naeslundii* 398A cells were suspended for 5 min in 1.0 ml of crystal violet diluted 1/100 and washed 7 times with buffer. The lower aqueous phase in the tubes containing the safranin-stained *S. oralis* droplets was replaced with 2.0 ml of a 1 : 1 mixture of the *A. naeslundii* suspension and buffer, and the droplets were rotated for 1 h. 2) To stain the streptococci for confocal microscopy, the *S. oralis*-coated droplets were rotated for 30 min with 10 μg/ml acridine orange in the aqueous phase and washed 3 times. They were then rotated for 1 h with the usual *A. naeslundii* suspension in coadhesion buffer until the aqueous phase was no longer turbid and washed again. The bound *A. naes-*

lundii cells were stained by indirect immunofluorescence using a 1/100 dilution of rabbit anti-*A. naeslundii* 398A whole-cell antiserum followed by 5 μg/ml rhodamine-conjugated anti-rabbit globulin, with intervening wash cycles. The samples were scanned with a Leica Fluovert Confocal Laser Scanning Microscope (Leica Lasertechnik, Heidelberg, Germany) using dual channel detection: one channel with an OG590 filter to detect rhodamine emissions and the other with an OG515 filter to detect acridine orange emissions. Twenty 2.0-μm optical sections in the x/y plane, scanned 4 times each, were reconstructed into a single image by an extended focus reconstruction program, and stereo pairs were created optically. 3) Samples of *S. oralis*-coated droplets and droplets with bound *A. naeslundii* were also fixed and embedded for conventional ultrathin sectioning and transmission electron microscopy.

The degree of coadhesion was calculated as the percent decrease in turbidity of the aqueous phase when compared with the initial reading. The rate of coadhesion was also calculated from the slopes of log [(at/a0) × 100] versus time, where "at" is the absorbance$_{550}$ for time "t" and "a0" is the absorbance$_{550}$ for time "0". These data were used to generate regression curves for rate in response to inhibitor concentration. Experiments were repeated at least twice; representative data are illustrated.

Inhibitors considered nonspecific included the detergents Tween 20 and sodium dodecyl sulfate (SDS), the hydrophobicity-inhibiting proteins bovine serum albumin (BSA) and defatted BSA and the salts LiCl and KCl, which would be expected to affect both electrostatic and hydrophobic interactions. The coadhesion assay was also tested over a pH range from pH 5.5 to pH 10. Lactose was selected as the specific inhibitor because it is a soluble β-galactoside used routinely to inhibit and reverse streptococcal – *A. naeslundii* coaggregation (7, 13). Its inhibitory concentrations were compared with that of β-methyl and α-methyl galactoside, with glucose included as a control. All reactions were done in the presence of calcium, as it is required for the lactose-sensitive lectin-like functions of *A. naeslundii* (4). The inhibitors were added singly or in combination to the mixture over a wide range of concentrations to determine the effect on coadhesion rate, and they were also added to *S. oralis*-coated droplets with preattached *A. naeslundii* cells to determine their effect on the *A. naeslundii* desorption rate. For assays combining the inhibitors, the lactose concentration was adjusted above and below 60 mM (the concentration closest to halving the coadhesion rate [when coadhesion rate data were expressed by simple regression curve] while achieving 100% inhibition) and combined with a broad range of concentrations for the salts.

Because Tween 20 and SDS emulsified the hexadecane droplets over the concentration range used, inhibition experiments with these detergents and BSA were conducted by coaggregation on glass slides, as reported previously (9). One drop each of *S. oralis* 34 and *A. naeslundii* 398A suspensions were mixed in wells of a flocculation slide and agitated at ambient temperature on a TekTator V serological rotator (American Hospital Supply Corp., Evanston, IL) at a speed of 90 rpm for 5 min. The degree of coaggregation was scored visually, with 0 representing no visible aggregation and 4+ assigned to aggregates yielding a single large clump. BSA and defatted BSA were tested at final concentrations ranging from 0.33 mg/ml to 1.67 mg/ml. SDS was tested at final concentrations ranging from 0.017% to 0.33% (wt/vol) and Tween 20 from 0.017% to 0.33% (vol/vol).

Desorption of *A. naeslundii* from *S. oralis*-coated hexadecane droplets

S. oralis-coated droplets with adsorbed *A. naeslundii* cells were washed by replacing the *A. naeslundii* suspension remaining in the aqueous phase with fresh, bacteria-free coaggregation buffer and then replacing it again with new buffer containing various concentrations of the inhibitors or control solutions. Lactose and glucose were tested over a concentration range of 10–100 mM. LiCl and KCl were tested over a range of 100–500 mM. For experiments using a combination of lactose and one of the salts, the lactose concentration was held constant at 60 mM. Tween 20 and SDS were also tested by incorporating them in the aqueous phase at concentrations 0.04–0.75% (wt/vol and vol/vol respectively) and gently rotating the tubes. The rate of desorption of bacteria was determined by measuring the increase in turbidity of the aqueous phase with time. Samples of the bacteria in the aqueous phase were stained and examined by light microscopy to determine whether *A. naeslundii* alone or both species desorbed.

Results

Coadhesion experiments

Both *A. naeslundii* 398A and *S. oralis* 34 adhered readily to *n*-hexadecane, in-

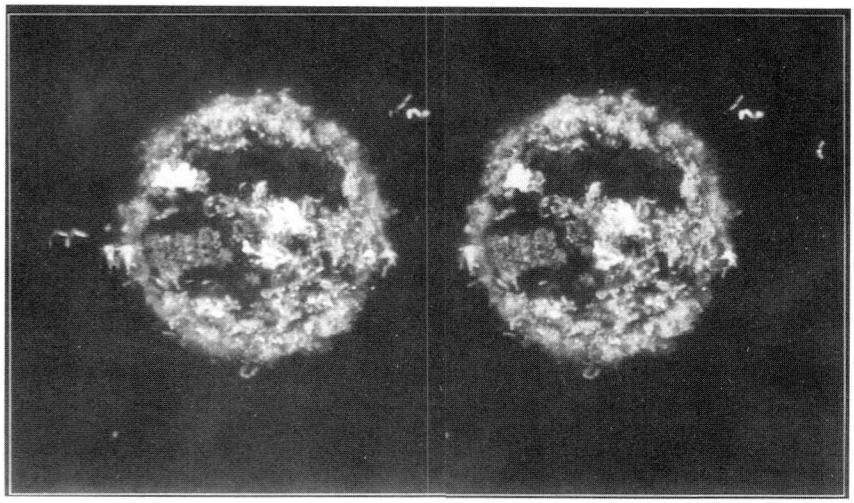

Fig. 1. Stereo pair of reconstructed confocal microscopy image of a *Streptococcus oralis* 34-coated hexadecane droplet following coadhesion assay with *Actinomyces naeslundii* WVU 398A. Adherent streptococci appear as small, light gray round cells, bound directly to the droplet surface. *A. naeslundii* cells, seen as brightly fluorescent rod-shaped bacteria due to adsorbed rhodamine-conjugated second antibody, adhered only to the streptococci, not to the bacteria-free hexadecane. The distribution of the bound bacteria is best seen in 3 dimensions by using a stereo viewer centered over the figure.

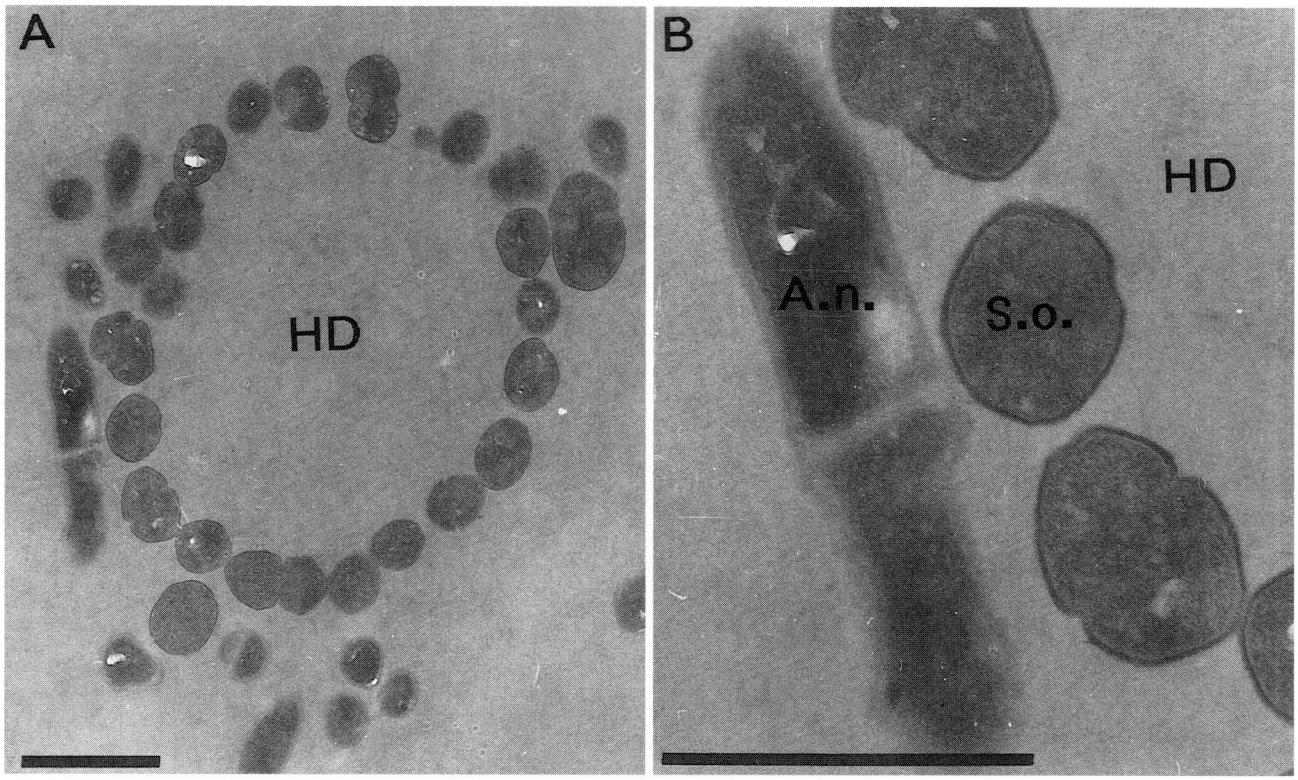

Fig. 2. Transmission electron photomicrographs of ultrathin sectioned *A. naeslundii* (A.n.) adhering to *S. oralis* (S.o.)-coated hexadecane (HD) droplet, demonstrating **A)** a section through an intact droplet and **B)** the close association of the two species connected by an electron-dense material. Bar = 1.0 μm.

dicating that their surfaces contain hydrophobic moieties. *S. oralis*-coated hexadecane droplets remained stable for days at ambient temperature, except when mixed with detergents. Light microscopy of droplets revealed areas containing monolayers of contiguous *S. oralis* cells separated by areas of hexadecane free of bacteria. When examined by both light and laser confocal microscopy, differential staining confirmed that the *A. naeslundii* cells bound only to the *S. oralis* cells, not to the bacteria-free areas (Fig. 1). The microscopy observations were further supported by turbidity measurements for the control tubes that contained hexadecane but no *S. oralis*-coated droplets. The turbidity of the aqueous phase remained constant throughout the assay, indicating that few, if any, *A. naeslundii* cells bound to hexadecane at the interface while the tubes were rotated gently. Electron microscopy confirmed that the *A. naeslundii* cells bound directly to *S. oralis* via dense surface coatings of both species and that neither species penetrated or deformed the hexadecane droplets (Fig. 2).

The β-galactosides lactose and β-methyl-galactoside inhibited the adhesion of *A. naeslundii* 398A to the *S. oralis*-coated droplets compared with controls containing either no potential inhibitor, α-methyl galactoside or glucose (Fig. 3). The minimum lactose concentration yielding any inhibition in this assay was 2.0 mM. Fig. 4 illustrates the effect of lactose on coadhesion rate over a wide range of concentrations. The data could be expressed as either a single straight line, 2 lines of different slope or a sigmoidal curve, all with high R^2 values. A minimum final concentration of 60 mM lactose was sufficient to decrease the rate by approximately half yet maintain a level of inhibition close to 100% (Fig. 3, 4).

The potential inhibitors of hydrophobic interactions that could be used in the hexadecane droplet assay, BSA

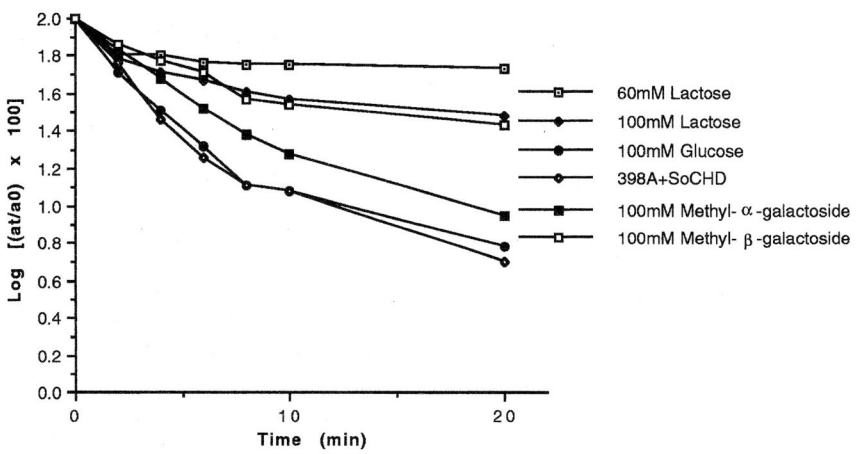

Fig. 3. The effect of galactosides and glucose control on the adhesion of *A. naeslundii* to *S. oralis*-coated hexadecane droplets. Data are expressed as the log percentage decrease in turbidity as a function of mixing time, where "a*t*" is the absorbance for time *t* and "a0"is the absorbance for time zero, just before mixing.

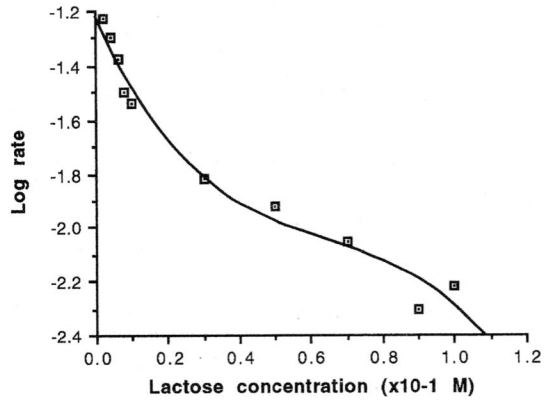

Fig. 4. Curve illustrating the rate of *A. naeslundii* adhesion to *S. oralis*-coated hexadecane droplets as a function of lactose concentration. A sigmoidal curve is shown, as it yielded the highest R^2 value ($R^2 = 0.975$). The data set could also be represented by a single, straight regression curve ($R^2 = 0.931$).

and defatted BSA, not only failed to inhibit coadhesion but actually increased the adhesion of *A. naeslundii* slightly at all concentrations (data not shown). They also failed to inhibit coaggregation in the slide assay (Table 1). SDS, but not Tween 20, was inhibitory in the slide coaggregation assay (Table 1). The salts LiCl and KCl yielded only minor inhibition of adhesion of *A. naeslundii* 398A to *S. oralis*-coated droplets, even at concentrations as high as 500 mM (Table 2). The salts achieved 50% coadhesion rate inhibition only when combined with >20 mM lactose. At lower lactose concentrations, even 500 mM of the salts failed to lower the coadhesion rate by more than 20%. There was no notable difference in coadhesion over a wide range of buffer pH from pH 5.5 to pH 10.

Desorption experiments

Adhesion of *A. naeslundii* to the *S. oralis*-coated droplets was rapidly reversible only when exposed to the specific inhibitor lactose in the aqueous phase (Fig. 5, 6). At concentrations 60 and 100 mM, lactose caused almost immediate desorption, reaching a plateau by 5 min (Fig. 5). Even at a concentration as low as 10 mM lactose, the desorption rate was more rapid than for the glucose control at 100 mM. No desorption was evident when the droplets were rotated with buffer as the aqueous phase. Neither LiCl (Fig. 6) nor KCl (data not shown; identical to LiCl) caused significant desorption of *A. naeslundii* from the *S. oralis*-coated droptlets, even at the highest concentration tested, 500 mM. Moreover, these salts did not noticeably alter the desorption rate for 60 mM lactose.

During the short period of the desorption experiment, some measurements of the effect of low concentrations of Tween 20 and SDS were possible. At the highest final concentration tested, 0.75%, SDS yielded desorption at a rate similar to lactose (Fig. 7). In contrast, Tween 20 had no effect. Microscopic examination of the bacteria desorbed by SDS yielded mixed morphotypes resembling both *A. naeslundii* and *S. oralis*, whereas bacteria desorbed by lactose were virtually all of an *Actinomyces* morphotype. Visual inspection of the separated phases indicated that SDS destabilized the hexadecane droplets.

Discussion

Data from the coadhesion assay used in this investigation support the hypothesis that stereospecific, β-galactoside-sensitive interactions have a greater impact than nonspecific interactions on the adhesion of *A. naeslundii* to *S. oralis*. The selectivity of *A. naeslundii* adhering to *S. oralis* cells rather than directly to hexadecane at the hydrocarbon-aqueous interface might represent the most graphic example of rapid attraction due to the avidity of specific interbacterial recognition mechanisms. Coadhesion was inhibitable and reversible at very low concentrations of lactose. In contrast, even high concentrations of salts known to disrupt hydrophobic interactions and to alter electrostatic interactions were ineffective unless combined with lactose well above its minimum inhibitory concentration. Moreover, coadhesion was not affected over the pH usually encountered *in vivo*. BSA, which is known to prevent hydrophobic and perhaps other nonspecific interactions and is often added to biological assays as a nonspecific blocker, had no inhibitory effect on either adhesion of *A. naeslundii* to *S. oralis* cells bound to hexadecane droplets or coaggregation between cells of the 2 species. Indeed, adding BSA actually increased the number of *A. naeslundii* cells bound.

The results do not fully negate the importance of nonspecific interactions in the surface attraction between *A. naeslundii* and *S. oralis*. Our obser-

Table 1. Slide coaggregation, *A. naeslundii* 398A and *S. oralis* 34

Bacteria	Test agent (final concentration)		Coaggregation score
S. oralis 34	Buffer		0
A. naeslundii 398A	Buffer		0
S. oralis and *A. naeslundii*	Buffer		4+
S. oralis and *A. naeslundii*	BSA	0.33 mg/ml	4+
		0.67 mg/ml	4+
		1.67 mg/ml	4+
	Defatted BSA	0.33 mg/ml	4+
		0.67 mg/ml	4+
		1.67 mg/ml	4+
	SDS	0.017%	2+
		0.003%	2+
		0.067%	1+
		0.167%	0
		0.333%	0
	Tween 20	0.017%	4+
		0.033%	4+
		0.067%	4+
		0.167%	4+
		0.333%	4+

Table 2. The effect of salts on the rate of coadhesion

	Coadhesion rate ($\times 10^{-2}$)[a] Final lactose concentration			
	60 mM	20 mM	2 mM	0 mM
Controls				
	1.49	1.37	2.53	2.95[b]
KCl (final concentration)				
10 mM	1.05	1.14	2.66	2.84
100 mM	0.64	0.91	2.30	2.14
200 mM	0.47	0.84	2.13	2.45
500 mM	0.24	0.94	2.24	2.39
LiCl (final concentration)				
10 mM	1.07	1.32	2.55	2.86
100 mM	0.81	1.05	2.57	2.52
200 mM	0.62	1.09	2.31	2.40
500 mM	0.40	0.74	2.44	2.44

[a] Coadhesion rate is defined as the slope of the curve expressing log $[(at/a0] \times 100)$ as a function of time, where "a*t*" is the absorbance for time "*t*" and "a0" the absorbance for time "0". [b] In different experimental runs this value ranged from 2.95 to 3.04.

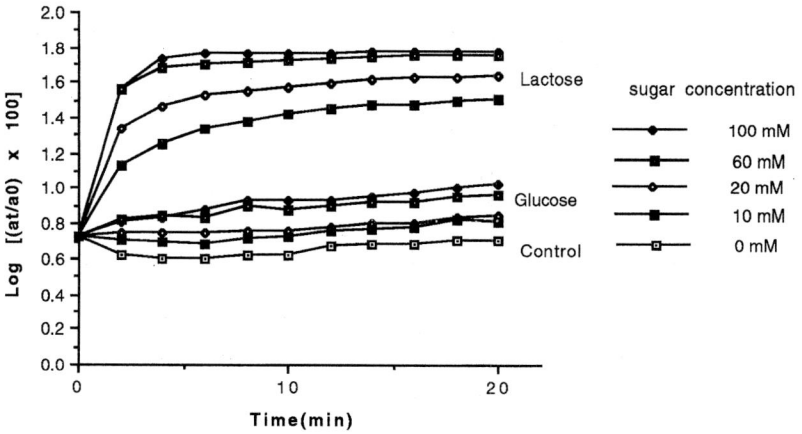

Fig. 5. The effect of various concentrations of lactose and the glucose control on the desorption of *A. naeslundii* from *S. oralis*-coated hexadecane droplets. Data are expressed as the log percentage increase in turbidity as a function of mixing time, where "a*t*" is the absorbance for time *t* and "a0" is the absorbance for time zero, just after replacing the *A. naeslundii* aqueous suspension with fresh bacteria-free buffer.

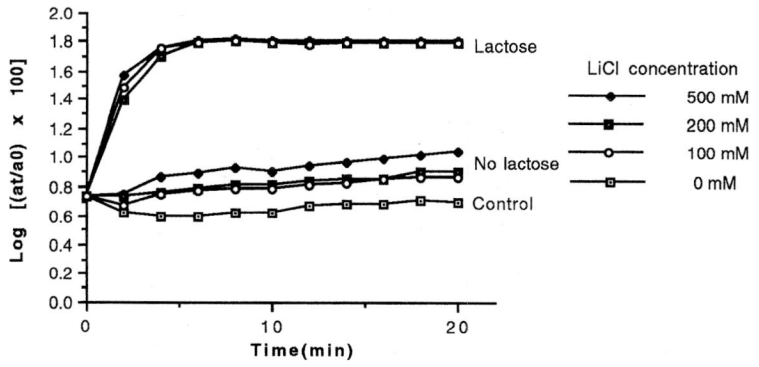

Fig. 6. The effect of various concentrations of LiCl with and without 60 mM lactose on the desorption of *A. naeslundii* from *S. oralis*-coated hexadecane droplets. Data are expressed as in Fig. 5.

vations confirm a previous report by McIntire et al. (13) that such amphipathic agents as SDS, singly and in combination with β-galactosides, reduce the degree of coaggregation between *A. naeslundii* (classified then as *Actinomyces viscosus*) T14V and *S. oralis* (classified then as *Streptococcus sanguis*) 34. While SDS clearly inhibited coaggregation by the slide test, its effect on the kinetics of coadhesion could not be determined because it disrupted the *S. oralis*-coated droplets. Therefore, it is difficult to differentiate whether inhibition derived solely from its interfering with attractive forces between the *S. oralis* and *A. naeslundii* cells. It is conceivable that detergent effects would be more significant in preventing or disrupting the homotypic adhesion between like bacterial cells than the heterotypic coadhesion between cells of different species. Our photomicrographs of droplets show that, whereas the *S. oralis* cells are densely crowded and in contact, the *A. naeslundii* cells do not adsorb closely enough together to establish avid lateral contacts with each other. The more generalized effect of SDS on homotypic adhesion is supported by the microscopic observation that SDS released both *A. naeslundii* and *S. oralis* from the droplets, whereas lactose released bacteria predominantly of an *Actinomyces* morphotype. Thus, results with inhibitors in coadhesion assays using a support surface might better represent the nature of coadhesive interactions important in establishing a sparsely populated biofilm or in selectively attracting a new species to the biofilm from the surrounding fluid suspension. Moreover, interpretation of inhibition results might be clearer in coadhesion than in coaggregation assays, because the former are amenable to analysis of both rate kinetics and endpoint.

Alternatively, the effects of "nonspecific" inhibitors, such as BSA or detergents, might be more specific than expected. In our laboratory's study of *P. gingivalis-A. viscosus* coadhesion, BSA and defatted BSA altered the slope of the coadhesion rate curve, but they did not totally inhibit the reaction (15). Subsequently, we found that several other proteins also prevented coadhesion, and we concluded that BSA was an inhibitor possibly because it served as a specific substrate for cell-associated proteases or other

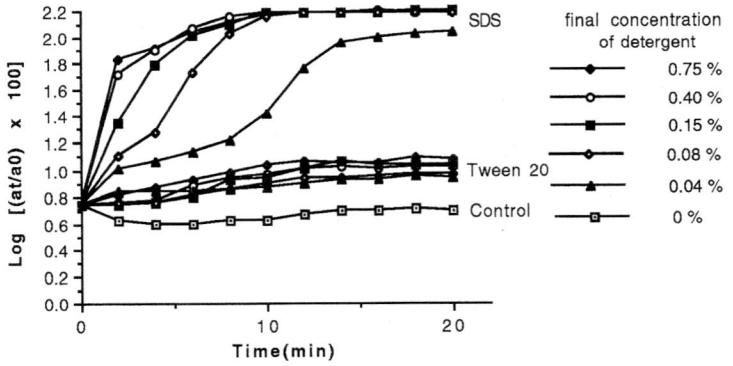

Fig. 7. The effect of various concentrations of SDS and Tween 20 on the desorption of bacteria from *S. oralis*-coated hexadecane droplets. Data are expressed as in Fig. 5.

specific binding molecules on *P. gingivalis* (5). Whether this pertains to *A. naeslundii-S. oralis* interactions remains an open question. Moreover, amphipathic molecules classified as detergents might have other nondetergent biological effects. Both our findings and the McIntire et al. coaggregation inhibition results demonstrate differences for SDS and Tween compounds. Indeed, McIntire et al. concluded that SDS inhibition of *A. viscosus-S. sanguis* coaggregation was not due to an ordinary detergent effect but due to the binding of SDS to the *A. viscosus* lectin in close proximity to the galactoside-combining locus (13). That is, it interfered with a stereospecific interaction.

KCl and LiCl had rather similar but limited inhibitory effects. Very high concentrations of these salts were required to alter the coadhesion rate, and they did not cause desorption. Lower salt concentrations affected coadhesion only in the presence of much lower concentrations of the specific inhibitor lactose. The effect of ionic concentration was much less than that previously reported for the adhesion of *A. viscosus* (20), and the effect of chaotropic LiCl was much less than that reported for the adhesion of *S. sanguis* to saliva-coated hydroxyapatite (14, 20). Our results suggest that the salts might cause a minor charge effect which impacts on β-galactoside inhibition. Despite ample evidence that hydrophobicity is crucial for cooperativity in adhesion of oral streptococci to hydroxyapatite, our data for *S. oralis* 34 coadhesion with *A. naeslundii* 398A do not fully satisfy all the criteria for a multiple class binding mechanism as cited by Doyle et al. (2). Yet curves generated from data expressing the relationship between lactose concentration and rate suggest that specific coadhesion interactions between the bacteria are more complex than if binding interactions between the adhesion molecules themselves were studied in isolation. Nevertheless, it is evident that, in this *in vitro* system, stereospecific interactions dominate coadhesion, supporting recent evidence that lectin specificity may be a significant determinant of the intraoral distribution of *Actinomyces* strains (19) and their establishment on surfaces coated with coaggregation-positive streptococci (17). Although pH, ionic concentration and chaotropic agents should destabilize noncovalent interactions, the multiple lectin-carbohydrate interactions between whole cells of *A. naeslundii* and *S. oralis* must be so avid that conditions way beyond the physiologic range seem necessary to affect coadhesion. It follows that if preventing or disrupting selectively targeted coaggregation reactions in dental plaque is to become an eventual therapeutic goal, then incorporating specific inhibiting or desorbing agents might be advantageous.

Acknowledgements

This investigation was supported by grant MT-5619 from the Medical Research Council of Canada. H.V. was the recipient of an MRC summer studentship.

References

1. Ciardi JE, McCray GFA, Kolenbrander PE, Lau A. Cell-to-cell interaction of *Streptococcus sanguis* and *Propionibacterium acnes* on saliva-coated hydroxyapatite. Infect Immun 1987: **55**: 1441–1446.
2. Doyle RJ, Rosenberg M, Drake D. Hydrophobicity of oral bacteria. In: Doyle RJ, Rosenberg M, ed. Microbial cell surface hydrophobicity. Washington, DC: Am Soc Microbiol, 1990: 387–419.
3. Ellen RP. Oral colonization by gram-positive bacteria significant to periodontal disease. In: Genco RJ, Mergenhagen SE, ed. Host-parasite interactions in periodontal diseases. Washington, DC: Am Soc Microbiol, 1982: 98–111.
4. Ellen RP, Fillery ED, Chan KH, Grove DA. Sialidase-enhanced lectin-like mechanism for *Actinomyces viscosus* and *Actinomyces naeslundii* hemagglutination. Infect Immun 1980: **27:** 335–343.
5. Ellen RP, Song M, Buivids IA. Inhibition of *Actinomyces viscosus-Porphyromonas gingivalis* coadhesion by trypsin and other proteins. Oral Microbiol Immunol 1992: **7**: 198–203.
6. Kolenbrander PE, Ganeshkumar N, Cassels FJ, Hughes CV. Coaggregation: specific adherence among human oral plaque bacteria. FASEB J 1993: **7**: 406–413.
7. Kolenbrander PE, Williams BL. Lactose-reversible coaggregation between oral actinomycetes and *Streptococcus sanguis*. Infect Immun 1981: **33**: 95–102.
8. Komiyama K, Gibbons RJ. Inhibition of lactose-reversible adherence between *Actinomyces viscosus* and oral streptococci by salivary components. Caries Res 1984: **18**: 193–200.
9. Li J, Ellen RP. Coaggregation of *Porphyromonas* (*Bacteroides*) gingivalis, other species of *Bacteroides*, and *Actinomyces viscosus*: methodological evaluation. J Microbiol Methods 1990: **12**: 91–96.
10. Li J, Ellen RP. Relative adherence of *Bacteroides* species and strains to *Actinomyces viscosus* on saliva-coated hydroxyapatite. J Dent Res 1989: **68**: 1309–1312.
11. Li J, Ellen RP, Hoover CI, Felton JR. Association of proteases of *Porphyromonas* (*Bacteroides*) *gingivalis* with its adhesion to *Actinomyces viscosus*. J Dent Res 1991: **70**: 82–86.
12. Liljemark WF, Bloomquist CG, Bandt CL, Pihlstrom BL, Hinrichs JE, Wolff LF. Comparison of the distribution of *Actinomyces* in dental plaque on inserted enamel and natural tooth surfaces in periodontal health and disease. Oral Microbiol Immunol 1993: **8**: 5–15.
13. McIntire FC, Crosby LK, Vatter AE. Inhibitors of coaggregation between *Actinomyces viscosus* T14 and *Streptococcus sanguis* 34: β-galactosides, related sugars, and anionic amphipathic compound. Infect Immun 1982: **36**: 371–378.
14. Nesbitt WE, Doyle RJ, Taylor KG. Hydrophobic interactions and the adherence of *Streptococcus sanguis* to hydroxylapatite. Infect Immun 1982: **38**: 637–644.

15. Rosenberg M, Buivids IA, Ellen RP. Adhesion of *Actinomyces viscosus* to *Porphyromonas* (*Bacteroides*) *gingivalis*-coated hexadecane droplets. J Bacteriol 1991: **173**: 2581–2589.
16. Schwarz S, Ellen RP, Grove DA. *Bacteroides gingivalis-Actinomyces viscosus* cohesive interactions measured by a quantitative binding assay. Infect Immun 1987: **55**: 2391–2397.
17. Skopek RJ, Liljemark WF, Bloomquist CG. Dental plaque development on defined streptococcal surfaces. Oral Microbiol Immunol 1993: **8**: 16–23.
18. Stinson MW, Safulko K, Levine MJ. Adherence of *Porphyromonas* (*Bacteroides*) *gingivalis* to *Streptococcus sanguis in vitro*. Infect Immun 1991: **59**: 102–108.
19. Strömberg N, Borén T. *Actinomyces* tissue specificity may depend on differences in receptor specificity for GalNAcβ-containing glycoconjugates. Infect Immun 1992: **60**: 3268–3277.
20. Wheeler TT, Clark WB, Lane MD, Grow TE. Influence of physicochemical parameters on adsorption of *Actinomyces viscosus* to hydroxyapatite surfaces. Infect Immun 1983: **39**: 1095–1101.